JCMM
Available online at
www.jcmm.ro
www.jcmm.org
Apoptosis Review Series

J. Cell. Mol. Med. Vol 9, No 2, 2005 pp. 345-359

Pathways of apoptosis and importance in development

Ciara Twomey, J.V. McCarthy *

Signal Transduction Laboratory, Biochemistry Department,
Biosciences Institute, University College Cork,
Cork, Ireland

Received: February 28, 2005; Accepted: March 19, 2005

Abstract

The elimination of cells by programmed cell death is a fundamental event in development where multicellular organisms regulate cell numbers or eliminate cells that are functionally redundant or potentially detrimental to the organism. The evolutionary conservation of the biochemical and genetic regulation of programmed cell death across species has allowed the genetic pathways of programmed cell death determined in lower species, such as the nematode *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster* to act as models to delineate the genetics and regulation of cell death in mammalian cells. These studies have identified cell autonomous and non-autonomous mechanisms that regulate of cell death and reveal that developmental cell death can either be a pre-determined cell fate or the consequence of insufficient cell interactions that normally promote cell survival.

Keywords: apoptosis - development - evolution - genetics - Drosophila melanogaster - Caenorabditis elegans - caspase - death receptor - Bcl-2 - IAP

Introduction

Extensive research has provided abundant information on the biochemical and molecular mechanisms that regulate cell growth, cell proliferation and cell death. The coordinated regulation of these events ensures normal embryonic development and tissue homeostasis. Increasing evidence from genetic studies in the fruitfly, *Drosophila melanogaster*, and murine models, indicate that the mechanisms which regulate cell proliferation are balanced against the regulation of cell death [1–3]. Developing tissues compete to survive and proliferate, and organ size is the result of a balance between cell proliferation and cell death, controlled by local cell environments which allow cells to divide, stop dividing, or die.

Physiological cell death, when initially described, was based on characteristic morpho-

* Correspondence to: J. V. McCARTHY,
Signal Transduction Laboratory, Biochemistry Department,
Biosciences Institute, University College Cork, Cork, Ireland.
Tel.: +353-21-490-1302:
Fax: +353-21-490-1382
E-mail: jv.mccarthy@ucc.ie

Reprinted from:
Journal of Cellular and Molecular Medicine

Published by:
the CMM Foundation

logical events that could be readily seen in dying cells. Later the term programmed cell death was employed to distinguish these genetically regulated types of cell death from necrosis, cell death resulting from cell injury [4]. Three primary types of physiological cell death were originally described based on morphological studies of developing vertebrate embryos [5] - heterophagy, autophagy and non-lysosomal cell death. Heterophagy is now widely known as apoptosis and occurs in isolated dying cells showing cell shrinkage, chromatin condensation, cell fragmentation into apoptotic bodies and eventual phagocytosis by neighbouring cells. Autophagic cell death is observed when groups of cells or entire tissues die. These cells do not rely on phagocytosis by neighbouring cells to degrade the dying cell but themselves contain cytoplasmic autophagic vacuoles that degrade the cell contents. Non-lysosomal cell death is not commonly observed in developing embryos.

The physiological role of cell death in mammalian development has been determined in elegant *in vivo* studies, based upon the generation and functional characterisation of transgenic gene overexpression and gene deletion murine models [6–9]. These models in part have also indicated a high degree of genetic redundancy in mammalian systems and have hindered definitive conclusions. With this in mind, researchers turned to lower organisms that were both developmentally characterised and genetically manipulable to provide model systems in which to study the developmental regulation of cell death. The nematode, *Caenorhabditis elegans,* undergoes a highly reproducible and regulated cell death which allows every cell to be followed during development [10, 11] and provided the first evidence for the genetic regulation of apoptosis [10]. The fruitfly *Drosophila melanogaster* provides a system of intermediate complexity between the nematode and mammals, sharing many mammalian components and pathways but having less redundancy, thereby allowing easier identification of specific functions [12–15]. Studies in less complex model organisms' benefit from the powerful genetics that can be used to generate transgenic lines that either removes gene function (gene knockout) or ectopically over express genes (transgenic animals). These systems allow the examination of genetic interactions and functional characterisation of novel genes using genetic screening methods. This review will concentrate on the conservation of the genetics and biochemical pathways leading to programmed cells death and developmental studies in *C. elegans*, *Drosophila* and murine models, which have enhanced our understanding of apoptosis in mammalian cells.

The genetics of developmental cell death in *C. elegans*

The nematode *C. elegans* has proved to be very useful for investigating how patterns of cell fates are established during animal development because it has a completely defined and largely invariant cell lineage [16, 17]. Namely, the division, differentiation and development fate of each individual cell follows a precise and predetermined pathway of development. Individual cells can be easily observed in live animals, comparative animal-to-animal studies are readily performed and *C. elegans* is well suited for genetic and molecular analysis. During the development of an adult *C. elegans*, 131 out of the total 1090 cells undertake autonomous cell death in a lineage-specific manner. Genetic studies in *C. elegans* have defined a variety of single-gene mutations that have specific effects on apoptosis [17]. Analysis of the genes defined by these mutations have revealed that apoptosis is an active process which requires the function of specific genes, some required to cause cell death while others protect cells from dying. The genetic dissection of this developmental cell death in *C. elegans* has lead to the identification of three groups of genes involved in this process. The first group of genes includes *ces-1* and *ces-2* (*ces*, cell-death specification) and affects the death of specific types of cells. The second group of genes affects most, if not all, of the 131 cells undergoing apoptosis, and is therefore defined as the core apoptotic pathway. These highly conserved regulators, which include four genes, *egl-1* (egl, egg-laying defective), *ced-3* (*ced,* cell-death abnormal), *ced-4*, and *ced-9* are central to developmental-apoptosis in *C. elegans* [10, 18]. The last group of genes, which includes *nuc-1* (*nuc*, nuclease) *ced-1, ced-2, ced-5, ced-6,*

ced-7, ced-10 and ced-12, is involved in the degradation of DNA and phagocytosis of apoptotic cells [19]. Among the three groups of cell-death genes, those involved in the execution phase of apoptosis have been most extensively studied, with biochemical studies suggesting that expres-

Table 1 Evolutionary conservation of sequence or function in cell death genes in different species.

	C. elegans	*Drosophila*	*Mammals*
Transcription	CES-1, CES-2		
Initiator caspases	CED-3	DRONC, DCP-2/DREDD, Dream	Caspases 1, 2, 8, 9, 10,12
Effector caspases	CSP-1, CSP-2	DRICE, DCP-1, DECAY, DAYDREAM	Caspases 3, 6, 7
Anti-apoptotic Bcl2 family			
Pro-apoptotic Bcl2 family	EGL1	Debcl/dBorg-1/d-Rob-1,	Bax, Box/Htd, Bod, Bad, Bim, Bmf, Hrk/DP5, Blk, Hip 3, BNip3/Nix, Puma, Noxa, Spike
Apaf-1	CED-4	d-Apaf-1/ Dark/ ARK, HAC 1	Apaf-1
IAP family		dIAP1/Thread,d–IAP2, DETERIN, dBruce	XIAP, cIAP1, cIAP2, NIAP, ILP2, ML-IAP/LIVIN, Bruce/ Apollan, SURVIVIN
IAP binding proteins		Reaper, Hid, Grim, Sickle	SMAC/DIABLO, Omi/HtrA2
Extrinsic pathway components		Death receptors and adaptor proteins ?	Death receptors and adaptor proteins
AIF **Endo G**	WAH-1 CSP-6	fAIF D. melanogaster CG8862 gene	AIF EndoG
Engulfment	Nuc-1, ced-1, ced-2, ced-5, ced-6, ced-7, ced-10, ced-12		SREC, ABC1, hCED6, ELMO, CRKII, DOCK180, RAC1

Table 2 Phenotypic comparison of transgenic or gene knockout models.

Gene	*Model*	*Mutation and phenotypic effect*
Murine:		
Caspase – 8	Knockout	In utero death, heart and haematopoietic defects
Caspase – 3	Knockout	Neuronal defects
Caspase – 9	Knockout	Unviable, neuronal defects
Bid	Knockout	Incapable of Fas-dependant apoptosis
Bak	Knockout	Normal phenotype
Bcl-x	Knockout	Unviable, neuronal defects
Apaf-1	Knockout	Usually not viable, neuronal defects, persistence of interdigital webs
p53	Knockout	Normal development but more vulnerable to tumours
FADD	Knockout	Embryonic lethality
Drosphila:		
DCP-1	Knockout	Larval lethality
Dredd	Loss of Function	Compromised immune response
Dronc	RNAi	Absence of PCD during development
dBorg 1	Knockout	Surplus glial cells
Reaper	Knockout	Absence of embryonic cell death
d-IAP1, d-IAP2	Over expression	Inhibit death induced by RHG proteins
C. elegans:		
EGL-1	Gain of Function	Excessive apoptosis
EGL-1	Loss of function	Absence of PCD
Ced 3	Loss of function	Absence of cell death
Ced 4	Loss of function	Abnormal cell lineage
Ced 9	Gain of Function	Prevention of PCD
Ced 9	Loss of function	Abnormal apoptosis

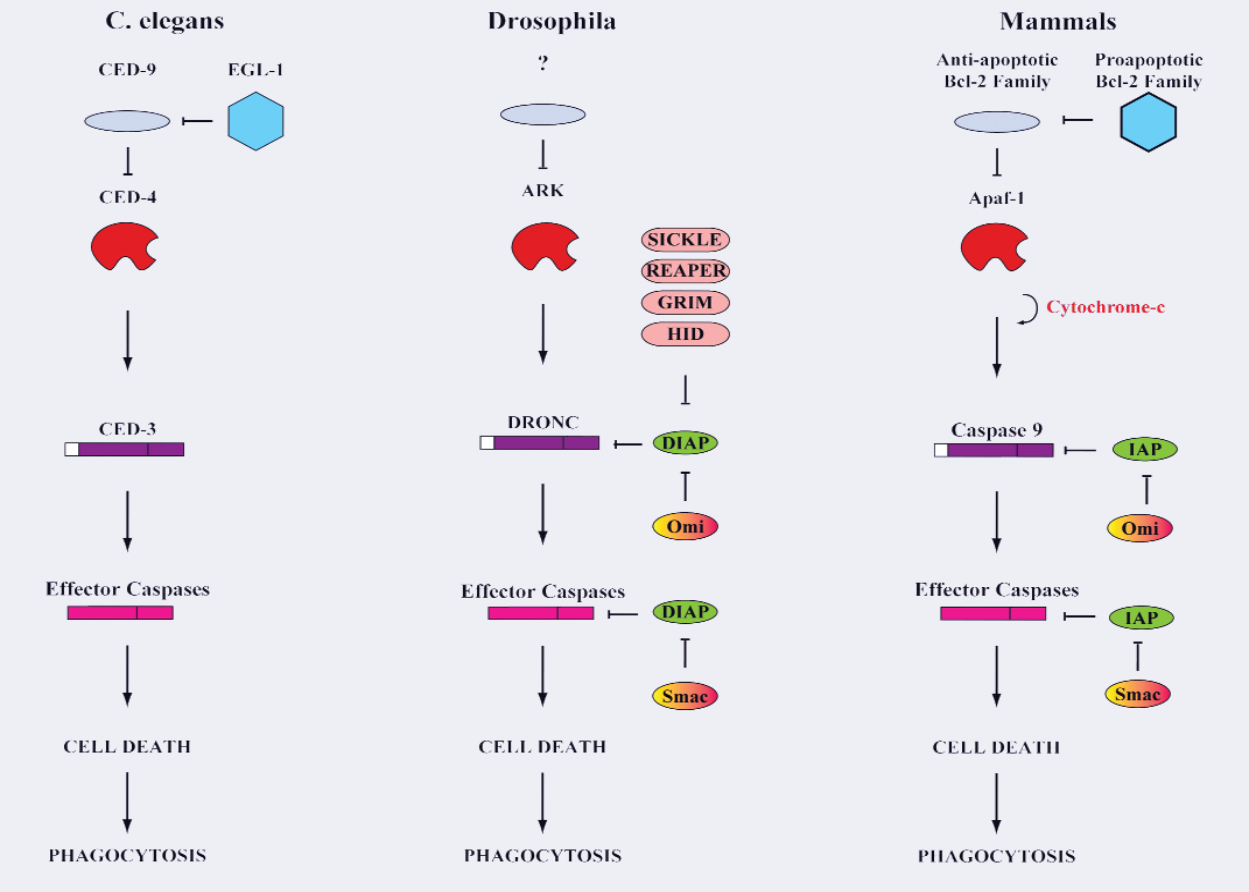

Fig. 1 Evolutionary conserved cell death pathways in C. elegans, Drosophila and mammals. Functional homologues of caspases and their regulators across species are indicated by the same colour. The mammalian proteins that participate in the intrinsic apoptosis pathway and homologous proteins in *C. elegans* and *Drosophila* are represented. In *C. elegans*, the initiator caspase, CED-3 is activated by CED-4 but inhibited by CED-9. In mammals the intrinsic pathway involves the translocation to mitochondria of pro-apoptotic Bcl-2 family members, which result in the release of mitochondrial cytochrome-c, oligomerisation of Apaf-1, activation of caspase-9 and subsequent activation of effector caspases. In mammals and *Drosophila* the inhibitor of apoptosis proteins (IAPs) bind to and inhibit activated caspases. The mitochondrial proteins Omi and Smac/Diablo, also interacts with the IAPs and prevents them from inhibiting caspase activation, thereby inducing apoptosis, whereas in *Drosophila* the RGH proteins, Sickle, Reaper, Grim and Hid can additionally suppress the IAP-mediated negative regulation of caspases.

sion of *egl-1*, *ced-3* and *ced-4* are required for the induction of apoptosis, whereas expression of *ced-9* is necessary to inhibit cell death [11].

Conservation and regulation of cell death

Observations of a common morphology of dying cells across species pointed towards a common basis for cell death and later the identification of conserved pathways and functional homology in the cell death machinery involved were elucidated. Research in apoptosis has demonstrated that even though core elements of the apoptotic machinery are widely conserved across species, animals with increased complexity possess additional proteins that control the precise execution of the apoptotic process [20, 21]. In an apparent reflection on increased animal size and complexity of systems such as the immune and nervous system there are greater numbers of relevant proteins in each func-

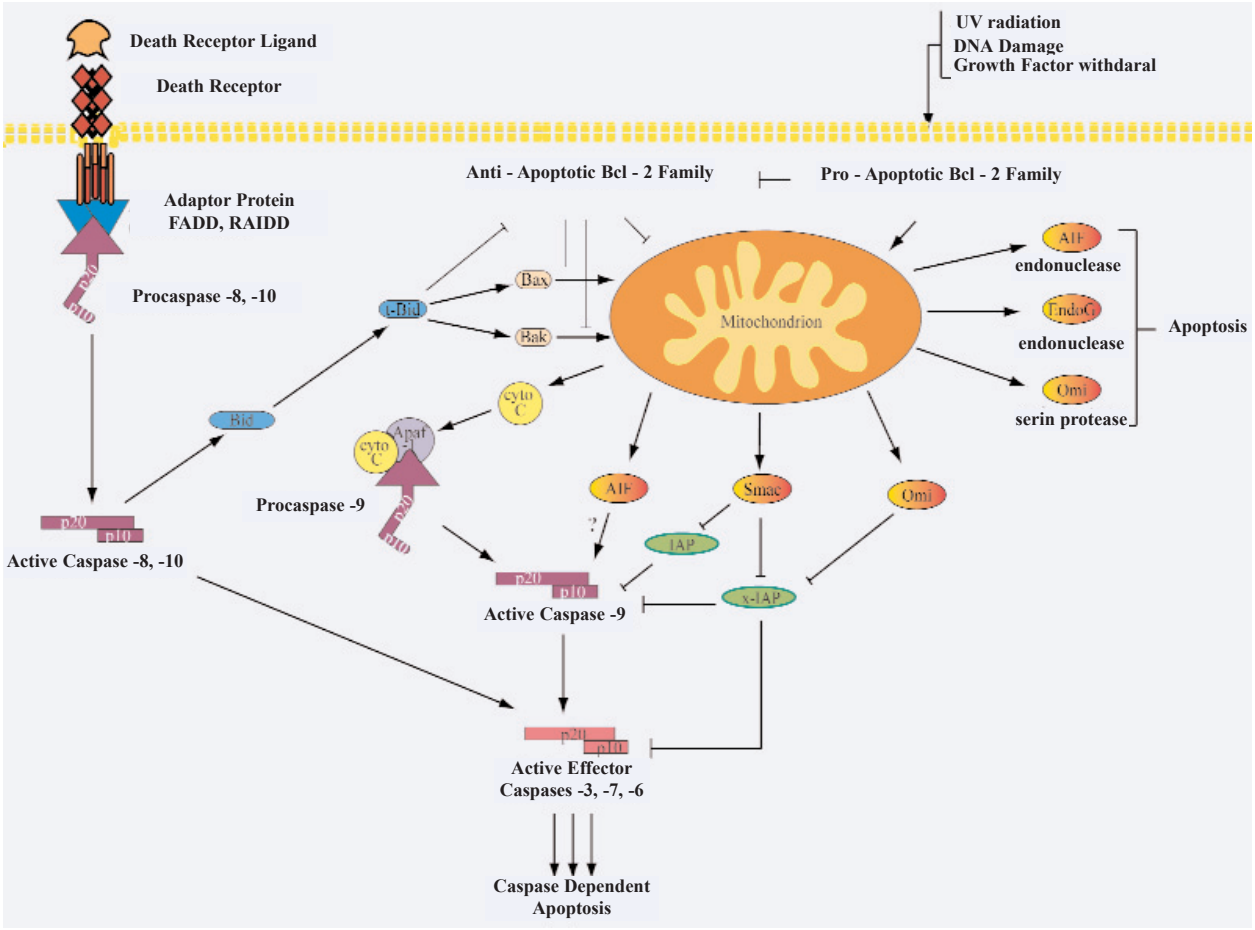

Fig. 2 Pathways leading to mammalian cell death. The extrinsic apoptosis pathway (left) is induced by death receptor ligand (TNF, Trail, FasL etc) which results in the recruitment and formation of a multiprotein complex DISC that includes the death-receptor, intracellular adaptor proteins (TRADD, FADD, RAIDD) and initiator caspases (procaspase 8), leading to autocaltalytic processing and activation of the initiator caspase. The intrinsic pathway (right) is initiated by the majority of apoptotic stimuli, including irradiation, cytotoxic drugs, granzyme B and DNA damage. Loss of mitochondrial membrane potential and release of pro-apoptotic cell death proteins (see text) results in the formation of another multiprotein complex, the apoptosome, that includes Apaf-1, cytochrome-c, ATP/dATP and the initiator caspase, procaspase 9, leading to autocatlaytic activation of caspase-9 and subsequent effector caspases. Pro- and anti-apoptotic bcl-2 homologues regulate release of pro-cell death mitochondrial proteins, while activity of caspases is negatively regulated by the IAPs. Smac and Omi promote caspase activation by antagonising the inhibitory effects of the IAPs, while AIF and EndoG contribute to caspase-independent cell death.

tional class though they appear to have differential involvement depending on the apoptotic stimulus (Table 1). This context-dependent participation is reflected in knockout and transgenic models - modulation of certain proteins are incompatible with life, others affect certain organs or systems in isolation while functional redundancy among the death machinery results in the absence of an obvious phenotype in others [6–8, 22] (Table 2).

ICE (interleukin-1-β-converting enzyme) was initially identified as a novel mammalian protease with an important role in inflammation responsible for the processing of pro-IL-1β to the active cytokine. Several years later it was shown that ICE was related to the protein product of the *C. elegans* death gene, *ced-3* [23]. This observation suggested that cystine proteases may be essential for the regulation of the cell death process and that the molec-

ular mechanisms of cell death might be highly conserved. Subsequently, gene interaction studies have defined a genetic pathway in *C. elegans* and have ordered the functions of *egl-1*, *ced-9, ced-3* and *ced-4* [24] (Fig. 1) and homologues of the nematode core apoptotic pathway genes have been identified and shown to be conserved throughout evolution, along with additional activators, effectors and inhibitors of cell death [1] [21, 25, 26] (Table 1). The *ced-3* gene encodes a defining member of a continuously growing family of specific cystine proteases termed caspases (*c*ystine-*asp*artate prote*ase*). Subsequent studies have lead to the identification of a total of fourteen mammalian caspases (caspase 1–14) [27]. Except caspsase-11 (murine), caspase-12 (murine) and caspase-13 (bovine), all other identified caspases are of human origin. In *Drosophila* seven caspases have been identified thus far: *Dcp-1, Dredd/Dcp-2, Drice, Dronc, Decay, Strica/Dream and Damm/Daydream* [14]. Phylogenetic analysis has revealed that the caspase family is broadly grouped into two major subfamilies, based on their proteolytic specificities and relation to either caspase-1 (ICE) which function in cytokine maturation or the mammalian homologues of CED-3 which function in programmed cell death [26]. The CED-3 like caspases are further subdivided into two groups, the initiator caspases and the effector caspases. Caspases are activated sequentially resulting in the activation of a caspase cascade and amplification of death signals. This pathway is initiated with autocatalytic activation of initiator caspases that in turn transmit the signal by cleaving and thereby activating the effector caspases. In humans the initiator caspases (pro-caspase 2, 8, 9, 10, and 12) and inflammatory caspases (pro-caspase 1, 4, 5, 11 and 13) contain long prodomains that contain distinct motifs, including the **d**eath **e**ffector **d**omain (DED) present in pro-caspase 8 and 10, and **ca**spase **r**ecruitment **d**omain (CARD) found in pro-caspase 1, 2, 4, 5 and 9, which are required for oligomerisation and autocatalytic activation of these caspases. Active initiator caspases transmit the proteolytic signal by cleaving the short-prodomain effector caspases (pro-caspase 3, 6 and 7).

Mammalian cells have two primary cell death-inducing signalling pathways by which caspases can be activated, the intrinsic and extrinsic cell death-inducing pathways [28–30] (Fig. 2). In contrast, cell death in C. elegans is confined to a linear intrinsic cell-death pathway [24]. Depolarisation of the mitochondria and release of mitochondrial proteins is central to the intrinsic pathway. In addition to compromising the integrity of the cells energy source, mitochondrial depolarisation allows the release of pro-apoptotic proteins such as cytochrome c into the cytosol. A cytosolic complex, the apoptosome, is then formed consisting of oligomerised Apaf-1 (apoptotic protease-activating factor 1), ATP/dATP, cytochrome c and the initiator caspase, pro-caspase-9 [25, 26]. Oligomerisation of Apaf-1 allows the recruitment and autocatalytic activation of caspase-9 and consequently the propagation of a death signal by proteolytic processing and activation of effector caspases [31]. The extrinsic pathway is activated through extracellular cues and so links the extracellular microenvironment to intracellular viability. Upon ligand-induced death-receptor oligomerisation, a multimeric Death Inducing Signalling Complex (DISC) is formed through homophilic interactions with intracellular adaptor proteins that mediate the autocatalytic activation of the relevant initiator caspases [29, 32, 33]. The subsequent activation of effector caspases leads to the eventual apoptotic demise of the cell [34]. Cross talk can occur between the two pathways where induction of the extrinsic pathway can lead to the subsequent activation of the intrinsic pathway and thereby amplification of the apoptotic stimulus [21, 26, 34].

Like the caspases, the evolutionary origins of the constituents of the apoptosome can be evolutionarily traced back to *C. elegans* and *Drosophila* (Fig. 1). The nematode *ced-4* gene functions in the activation of *ced-3* and only one mammalian homologue, *Apaf-1*, has been characterised to date, which like *ced-4* functions in the activation of caspases [35]. In *C. elegans*, the CED-4 protein is normally localised to the mitochondria through an interaction with CED-9, unless EGL-1 is expressed. If EGL-1 is expressed the interaction between CED-4 and CED-9 is disrupted and CED-4 translocates to the nuclear membrane where it activates CED-3, which subsequently induces apoptosis. Both CED-4 and APAF-1 require dATP for caspase activation but mammalian APAF-1 also requires mitochondrial cytochrome-c [31]. *Drosophila* has recently been shown to have a *ced-4/Apaf-1* homologue, *Ark*, (previously called *dapaf-1, Dark, hac-1*). In *Drosophila*,

no conclusive evidence has been found that demonstrates a requirement for cytochrome c, however, similar to APAF-1 and CED-4, loss of function Ark mutants have reduced developmental apoptosis [36].

The nematode CED-9 and EGL-1 proteins belong to a growing family of proteins with homology to the mammalian protein Bcl-2 [28, 37, 38]. Members of the *bcl-2* gene family have an important role as sensors that control the occurrence of apoptosis [39]. This family is functionally divided into pro-apoptotic (Bax, Bak, Bok, Bad, Bid, Bim, Bmf, Hrk, Nbk, Bnip3, Noxa and Puma) and anti-apoptotic (*e.g.* Bcl-2 and Bcl-x_L) subgroups. CED-9 is homologous to the anti-apoptotic subgroup, while EGL-1 has mammalian counterparts in the pro-apoptotic protein group. Structural and functional analyses of Bcl-2 family members have identified four homologous regions, BH1, BH2, BH3 and BH4, (where BH means Bcl-2 homology) which are critical for function and protein-protein interactions. Based on these studies the Bcl-2 family members can be further subdivided into at least three distinct subfamilies, the Bcl-2 subfamily, Bax subfamily and the BH3-only proteins [38]. All cellular anti-apoptotic Bcl-2 homologues possess a BH1, BH2 and BH4 domain. In contrast, *C. elegans* CED-9 shows high sequence homology only in the BH1 and BH2 domains, while BH3 and BH4 domain functions are executed by non-homologous domains of the protein. Members of the Bcl-2 family can interact with each other, and these interactions are mediated through the BH1, BH2 and most importantly the BH3 domain. Deletion of the BH1, BH2 or BH3 domains of Bcl-2 impairs its ability to suppress apoptosis in mammalian cells. All pro-apoptotic family members contain the highly conserved BH3 domain and are subdivided into the Bax homologues (Bax, Bak, and Bok) and the BH3-only homologues that have only the BH3 domain (Bad, Bid, Bim, Bmf, Hrk, Nbk, Bnip3, Noxa and Puma). Mutation of the BH3 domain eliminates the apoptotic function of these Bcl-2 homologues [38].

In addition to regulating activation of CED-4/APAF-1, Bcl-2 family members have been shown to play a pivotal role in the regulation of mitochondrial homeostasis and release of pro-apoptotic factors such as AIF (apoptosis inducing factor), Smac/Diablo (second mitochondria derived activator of caspase/direct IAP binding protein with low pI), Omi/HtrA2 (high temperature requirement A2), Endonuclease-G and cytochrome-c, which mediate various aspects of apoptosis in mammalian cells [28, 40]. These proteins have been shown to function through caspase-dependent pathways (Smac/Diablo and cytochrome-c), caspase-independent pathways (AIF and Endonuclease-G), or both (Omi/HtrA2). The nematode homologues of Endo-G and AIF, CSP-6 and WAH-1 respectively, promote apoptotic DNA degradation and represent some of the mitochondrial proteins implicated in invertebrate apoptosis [41]. Thus Endo-G/CSP-6 and AIF/ WAH-1 provide another example of a single conserved apoptotic-signalling pathway between vertebrates and invertebrates.

Following the lead of studies in *C. elegans*, similar genetic studies in *Drosophila* have also validated the conservation of apoptosis signalling events through the identification of several additional regulators (Fig. 1). A region of the *Drosophila* genome that contains four genes, *sickle, reaper*, *grim* and *head involution defective (hid)* has been shown to be essential for virtually all apoptosis during *Drosophila* embryogenesis and development [14, 42, 43]. These genes have minimal sequence homology to any known mammalian proteins apart from a small region of 15 amino acids shared by all four genes. Evidence for the existence of vertebrate homologues of these genes come from the observations that these proteins are able to functionally interact with a family of inhibitors of apoptosis, the IAPs [44]. Although it has no sequence homology with the *Drosophila* genes *reaper*, *grim* or *hid*, the mammalian protein Smac/Diablo, does share many functional similarities. Like Reaper, Grim and Hid, Smac/Diablo also interacts with the IAPs and prevents them from inhibiting caspase activation, thereby inducing apoptosis (Fig. 1). Furthermore, Smac/Diablo interacts with the BIR (*baculovirus* IAP repeat) domain of the IAPs, similar to, Reaper, Grim and Hid.

The IAPs were first identified in *baculovirus*, but have since been shown to be important regulators of apoptosis in *Drosophila* and mammals [44] (Fig. 1). IAPs are pro-survival proteins containing between 1 and 3 BIR (*baculovirus* IAP repeat) domains, Zn binding motifs responsible for caspase binding and inhibition. Eight are described in mammals (XIAP, cIAP1, cIAP2, ML-IAP/LIVIN, ILP2, NIAP, Bruce/Apollan and SURVIVIN) where the prototype XIAP is the most studied. XIAP has three BIR

domains, of which BIR3 is responsible for mediating binding to and inhibition of caspase 9 activity. The linker region between BIR1 and BIR2 is responsible for binding to and inhibition of the effector caspases 3 and 7. After the mitochondrial targeting sequence of Smac/Diablo has been cleaved, an IAP binding motif is exposed capable of binding BIR3 of XIAP and so competitively relieving its inhibition of caspase 9. It's thought that this interaction also results in steric hindrance preventing XIAP from binding the effector caspases and XIAP at the same time and so the same protein relieves effector and initiator caspase inhibition differentially. There are two analogous proteins in *Drosophila*, d-IAP1 and d-IAP2. The BIR1 domain of d-IAP1 binds-to and inhibits the effector caspase DRICE, while the BIR2 domain binds to and inhibits Dronc. The suppression of both can be relieved through the RHG proteins, which are functionally comparable to Smac/Diablo and have an N-terminal IAP binding motif. There is however a fundamental difference with regard to mode of action of mammalian and *Drosophila* IAPs [13]. d-IAP 1 is an ε3 ligase and as such triggers the ubiquitination and breakdown of bound Dronc without affecting its catalytic activity.

Caspase independent apoptosis

Initially appearing unconventional, caspase-independent apoptosis has been shown to occur in a range of cell-types [40]. The mitochondrion is an integrative organelle in terms of apoptosis, a meeting point of both caspase-dependent and -independent pathways. The release of intermembrane proteins is involved in both so the identity and inherent function of the proteins released are decisive for the death pathway employed. Three proteins involved in caspase-independent apoptosis, AIF, Omi/HtrA2 and Endo G are conserved from bacteria to mammals signifying a preserved alternative mechanism of apoptosis. All are located in the mitochondria and are reliant on mitochondrial depolarisation to exert their pro-apoptotic activity [40]. Omi/HtrA2, after auto-proteolytic N-terminal processing, has high homology to the IAP binding proteins found in *Drosophila* and mammals. It can sequester and thereby inhibit the action of IAPs [45], though this has debatable physiological relevance since it only occurs at high concentrations. AIF, in healthy mammalian cells, is thought to function in the cells mitochondrial processes and as an endonuclease in apoptosis [46]. A DNA binding domain indispensable for its endonuclease activity is present though it has been hypothesised that AIF employs the action of a separate endonuclease to condense chromatin and cleave DNA. In addition it is thought to produce reactive oxygen species and so result in cytotoxicity, though there are reports of it inducing release of caspase 9 and so, like Omi/HtrA2, it may play a dual role [46, 47]. AIF has been shown to be sufficient and necessary for cavitation in embryoid bodies whereas members of the intrinsic pathway are dispensable. EndoG is an inner-mitochondrial/matrix associated protein and its RNAse activity is normally exploited for mitochondrial DNA replication. During cell death EndoG functions as an endonuclease. Evidence from transgenic mice implies that EndoG activity in apoptosis is reliant on localisation as opposed to relief of inhibition. In *C. elegans* CSP-6 (Endo G) and WAH-1 (AIF) collaborate to cleave DNA.

Developmental regulation of cell death

While *in vitro* studies have indicated a detailed and complex regulation of the genetics and biochemical pathways that control programmed cell death, our knowledge of cell death during development primarily comes from comparative studies of several model organisms *C. elegans*, *Drosophila* and mammals [1, 9, 10, 13, 15, 16, 20]. These genetic and developmental studies have indicated that physiological cell death occurs for many reasons during development, contributing to the regulation of cell number and organ size, elimination of superfluous structures, sculpting of tissues, and elimination of abnormal or aged cells [1, 2, 9]. These studies have also identified cell autonomous and non-autonomous mechanisms that regulate of cell death and reveal that developmental cell death can either be a pre-determined cell fate (autonomous) or the

consequence of insufficient cell interactions that normally promote cell survival (non-autonomous). There are two ways in which autonomous cell death can be initiated. First, apoptosis can be triggered by an underlying physiological cellular defect, such as DNA damage or a defect in differentiation. Second, developmental apoptosis can be viewed as a cell fate and occurs as a natural consequence of the normal process of terminal differentiation. In this way autonomous cell death is similar in a number of ways to adopting a state of differentiation, such as becoming a neuron or muscle cell. In *Drosophila* and mammals both autonomous and non-autonomous forms of cell death occur while in *C. elegans* very few cell deaths depend upon cell interactions but many are cell-autonomous [14, 48]. Like other developmental fates in *C. elegans*, autonomous cell death occurs throughout the cell lineage with the majority of cell deaths occurring during the developmental period when most other cells terminally differentiate. Similar to other cell fates in *C. elegans*, developmental apoptosis is observed in an invariant pattern with the same lineally equivalent cells undergoing apoptosis from animal to animal [16, 17]. This reproducibility in the cells that die suggests that this autonomous cell death is not a result of physiological cellular defects but rather indicates that developmental apoptosis is no different form developmental cell fates in general. In contrast, non-autonomous cell death is a mechanism for adjusting cell populations, which enables the emergence of architecture within an organ or tissue. It is now widely believed that some cells in vertebrates and *Drosophila* are fated to die unless their survival is maintained by cell-interactions and provision of survival factors or trophic factors produced from neighbouring cells [1, 3, 49]. Therefore, the integration of many cellular signals including extracellular survival factors [50, 51], cell-surface death receptors [29, 51], cell-lineage determinants [11], steroid hormones [13, 52], intracellular and extracellular stress signals [53] determine when cell death occurs during development. Studies have recently provided abundant information on how these signalling events initiate the regulated removal of specific cells by apoptosis.

Extracellular survival factors: Withdrawal of growth factor signals has long been recognised as an important mediator of cell death. During development, cell type specification and differentiation are tightly regulated by balanced rates of cell proliferation and cell death, which regulates cell numbers and ultimately organ and tissue sizes. During development some of the same molecules that regulate developmental processes also regulate the occurrence of cell death [3].

Studies involving development of the nervous systems in Drosophila and vertebrates have begun to provide a clearer picture of how extracellular survival factors regulate tissue development and homeostasis [54–56]. In vertebrates the most prominent of these are the neurotrophins and studies have now led to the trophic factor hypothesis [50]. In both *Drosophila* and vertebrates approximately 50% of cells in the developing nervous system undergo non-autonomous cell death, and this increases if interactions between cells are disrupted [54]. Neurons are produced in excess and the target tissue is a source of trophic factors, thus neurons that innervate the target tissue survive while misrouted neurons that fail to receive sufficient trophic factors die. In this way non-autonomous cell death controls cell survival and ensures proper innervation of tissues. [50, 57]. Likewise, non-autonomous cell death enables axonal guidance as neurons whose axons misroute either search for alternative routes to trophic factor producing targets that guarantee their survival, or die. Additionally, by adjusting neuronal and glial cell populations, non-autosomal cell death ensures correct myelination of neurons and enables the generation of complex structures, such as the retina [55]. Recently the mechanism underlying trophic factor signalling was determined using *Drosophila* development as a model system [58]. During the development of *Drosophila*, approximately ten midline glial cells are generated in each embryonic segment and function to separate axon tracts. Most of these glial cells die at a specific embryonic stage, dictated by the loss of SPITZ, a neuronal secreted ligand of the epidermal growth factor receptor (EGFR). In the presence of SPITZ, the EGFR initiates a Ras-mitogen activated protein kinase (MAPK) anti-apoptotic signalling pathway, which suppresses expression of the pro-apoptotic protein HID [59]. Therefore, loss of SPITZ signal enables HID to activate apoptosis by interacting with DIAP1 and releasing its inhibition of caspases [58]. Whether equivalent regulation by survival signals occurs in vertebrates remains to be determined.

The regulation of early neural cell death can be inferred from the function of factors such as bone morphogenetic proteins (BMPs), Wnts, fibroblast growth factors (FGFs), and Sonic Hedgehog (Shh), which regulate both early neural development and cell death occurring in other developmental processes [54]. BMP-mediated signalling is required for the maintenance of proliferation, mitotic exit, and subsequent differentiation of specific neural precursor cells. During development of the limb bud, where the interdigital necrotic zone undergoes apoptosis and segregation of neural crests, BMP has also been implicated in the regulation of cell death. Overexpression of BMP results in excessive cell death in both neural crests and limb buds and likewise overexpression of dominant negative forms of BMP receptors reduce the incidence of cell death in the limb bud. Similarly, Wnt signalling is required for survival and proliferation of neuronal crests, yet in a tissue-specific manner Wnt signalling can also mediated cell death. Like BMP, Wnts are involved in the regulation of cell death in the developing limb and neural crests. In the limb bud BMP and fibroblast growth factors (FGFs) stimulate the transcription of Dkk-1, a secreted Wnt-binding protein in dying cells [54].

Other growth factors such as insulin, insulin-like growth factors (IGFs) and FGFs are essential for maintaining survival of post-mitotic differentiated neurons. Insulin maintains neural precursors in the neuro-epithelium of the developing chick retina. Stimulation of *in vitro* cultures of chick retinal neuro-epithelium prevents apoptosis while *in vivo* antibody-mediated inhibition of insulin signalling increases the occurrence of cell death in the neuro-epithelium. Likewise, IGF-1 protects cultured neural precursors from apoptosis. It is thought that insulin and IGF-1 protection from apoptosis mediated by activation of Akt, which in turn phosphorylates and attenuates the effects of the pro-apoptotic Bcl-2 homologue, Bad.

Cell-lineage information: There are two forms of the nematode *C. elegans*, hermaphrodites and males. During development of the male, expression of the sex-determining gene *tra-1*, which encodes a zinc-finger transcription regulator, regulates apoptosis of hermaphrodite specific neurons. The Tra-1 protein represses transcription of the pro-apoptotic *egl-1* gene and thereby prevents induction of apoptosis by EGL-1 in hermaphrodites [60]. During male development EGL-1 induces apoptosis of hermaphrodite specific neurons by sequestering the anti-apoptotic protein CED-9. EGL-1 has significant sequence homology with other members of the Bcl-homology-3 domain (BH3) subfamily of pro-apoptotic Bcl-2 family members, suggesting that similar mechanisms may be utilised in other species.

Steroid hormones: As indicated earlier cell death can be akin to cell fate, where autonomous cell death can be inherited within a cell lineage like any other cell fate determinant. During *Drosophila* embryogenesis and development, apoptotic cells are observed throughout the embryo, particularly in the developing nervous system [13, 54, 55, 60]. In *Drosophila* peripheral nervous system the mechanosensory organ develops from one precursor that produces five progeny cells, of which the destined glial cell dies after birth. In this case, cell death is determined by the differential and asymmetric segregation of Numb at mitosis, which fails to prevent expression of pro-apoptotic genes in the cells lacking Numb expressing. Later during metamorphosis, abundant tissue and structural reorganisation involves extensive apoptosis that is regulated by specific changes in the concentration of the steroid hormones [14, 52]. The embryonic nervous system is destroyed before the adult system is made, and these events are triggered by the steroid hormone ecdysone, which upon systemic bursts induces death of cells expressing the ecdysone receptor. Further increases in ecdysone at the late larval/early pupal stage also induces cell death of larval tissues including the midgut and salivary glands, which are not needed in the adult [49, 55]. Ecdysone regulates both cell differentiation and cell death during insect metamorphosis by hierarchical transcriptional regulation of a number of genes, including the zinc finger family of transcription factors. These genes in turn regulate the transcription of a number of downstream genes. DRONC, a key apical caspase in *Drosophila*, is the only caspase known to be transcriptionally regulated by ecdysone during development [61, 62]. Reduced ecdysone concentrations also induce neuronal cell death by altering the transcription of the pro-apoptotic genes, *reaper, grim* and *hid* [60]. Cell population adjustments also take place during development of the *Drosophila* retina. The retina forms from the eye imaginal disc, which achieves a cer-

tain size before any differentiation occurs. The differentiation and survival fate of these cells is determined by non-autonomous cell-cell interactions, which dictate whether a neighbouring cell divides, differentiates or dies. The signalling pathway responsible for promoting cell survival and proliferation is the Ras-MAPK pathway triggered by the EGFR with its ligand SPITZ. Similarly in mammals, withdrawal of androgens induces apoptosis in the prostate gland and increased production of the male sex hormone testosterone induces apoptosis in mammary cells in males [1].

Developmental expression and modification of apoptotic components

Cell death is a tightly regulated process and aberrant regulation can lead to gross developmental defects or disease. It is important to maintain low levels of pro-apoptotic proteins in the cytoplasm under normal growth conditions but to rapidly induce their expression or activity when cells are programmed or stimulated to undergo apoptosis. For this reason, the function of many genes and proteins involved in apoptosis are developmentally controlled both at the transcriptional level and via several post-translational mechanisms. For example, at the protein level APAF-1 may be modulated by proteolytic cleavage, subcellular localization and by association with protein modifiers. Transcriptional regulation of *Apaf-1* has been implicated during development of the mammalian central nervous system.

Members of the *bcl-2*/*ced-9*/*egl-1* family are also modulated by transcriptional and post-translational mechanisms [53]. Egl1 is a BH3-only member of the Bcl-2 homologues and its expression is regulated by various transcription factors including, Tra-1A, Ces-1 and Ces-2. In *C. elegans*, expression of egl-1 in hermaphrodite specific neurons is normally repressed by the transcription factor Tra-1A in hermaphrodites, but not males [60]. In mammals, at least four *Bcl*-2 family members are subject to transcriptional control [53]. Noxa and Puma have been shown to be transcriptionally up regulated in response to p53, a transcription factor activated by DNA-damaging agents. During embryogenesis Bim and Hrk/DP5 expression are transcriptionally up regulated in response to activation of the Jun kinase pathway in trophic factor-deprived neurons and their levels peak at the time when these cells are committed to die. Phosphorylation-induced changes in protein conformation, which cause release from an inactive complex, increase affinity for formation of homo-dimers and hetero-dimers, and is also central to the post-translational regulation of members of the *bcl-2*/*ced-9*/*egl-1* family [53].

Drosophila hid is negatively regulated at the transcriptional and posttranscriptional levels by the EGFR - Ras - Raf - MAPK [58, 59]. By conducting genetic interaction studies in transgenic models of *Drosophila* it was also demonstrated that the EGFR pathway promotes cell survival by transcriptional repression of *hid* and that the MAPK phosphorylation sites in Hid are critical for this response [58]. Additional *in vitro* promoter analysis studies of *reaper, grim* and *hid* genes revealed that steroid hormones, developmental signals and growth factors modulate expression of these genes [12–14, 52].

In vivo gene manipulation studies

Developmental genetics in model systems, including *C. elegans* and *Drosophila* have helped to identify and order the components of apoptotic pathways. An even more complex network of apoptotic pathways has evolved in higher organisms that possess multiple homologues within each set of cell-death regulators. Comparison of transgenic or gene knockout mice with their wild type littermates has helped to elucidate mammalian apoptotic pathways and identify the principal effect of each cell death regulator, thereby enabling an assessment of the role played by specific genes in apoptosis during mammalian development (Table 2) [6–9].

The importance of Bcl-2 family homologues becomes apparent when the phenotype of mutant animals are characterised [7]. Targeted gene-knockout of the *bcl-2* gene in mouse models results in the occurrence of grey hair, polycystic kidney disease and lymphocytopenia [63]. In contrast, homozygous disruption of the anti-apoptotic *bcl-x* gene is embryonic lethal and coincides with massive apoptosis in post-mitotic immature neurons of

brain and spinal chord and in the haematopoietic system [64]. Homozygous disruption of *bcl-x* disrupts maturation of lymphocytes where the life span of immature nut not mature lymphocytes was shortened. These studies have allowed researchers to conclude that Bcl-x functions to support viability of immature cells during development of the nervous and haematopoietic systems [64]. Bax null animals have a milder phenotype, including hyperplasia of lymphocytes and ovarian granulosa cells and testicular degeneration. Similar studies have demonstrated that in the nervous system *Bax* is required for cell death following deprivation of neurotrophic factors [7]. *Bax* deficiency, like overexpression of *bcl-2* facilitates tumorigenesis, highlighting the importance of this family of proteins in maintaining tissue homeostasis and resistance to oncogenic transformation [7].

Apaf-1 null animals suffer a variety of developmental defects and either die *in utero* or shortly after birth [22]. *Apaf-1* null embryos exhibit severe craino-facial deformation, neural and brain overgrowth and retain inter-digital webs, all features of insufficient or compromised apoptotic cell death [22]. These studies demonstrate that *Apaf-1* is critical for apoptosis in the central nervous system and for normal brain development, though the role of *Apaf-1* in other forms of developmental apoptosis is still unclear. Unlike mice, *Drosophila* mutants that lack *Apaf-1* expression are viable, indicating the need for some caution when comparing developmental apoptotic processes between species, particularly *Drosophila* and humans.

Caspase-8 null mice die *in utero* as a result of defective development of heart muscle and display fewer haematopoietic progenitor cells, suggesting that the FADD/caspase-8 pathway is absolutely required for growth and development of specific cell types [6, 65, 66]. Similarly, animals with mutations in *caspase-3* and *caspase-9* die early in development and show defects in apoptosis in the nervous system resulting in brain overgrowth [9, 67, 68]. From these studies it is evident that cell death is an important component of development in the nervous system, brain and cardiovascular systems but more detailed studies are required to understand the precise role that dying cells have in the formation of these structures and systems.

Conclusions

Normal development is tightly regulated by cell division and apoptotic cell death. Over the past decade we have seen a tremendous expansion in our knowledge of the genetic and biochemical processes responsible for the activation, regulation and mechanisms of physiological cell death. Developmental genetics in model systems, including the nematode, fruitfly and mouse have helped to identify and order the components of apoptotic pathways. These studies revealed many similarities but also differences between nematodes, flies and mammals, demonstrating the apoptotic pathway is conserved across these species however the precise mechanisms that control the regulation of these events are not. Due to the close association between aberrant cell death and diseases (cancer and chronic degenerative diseases), the therapeutic modulation of apoptosis has become an area of intense research, but with this comes the demand for a more thorough understanding of apoptosis in whole organisms. In the future, determining how cell death is integrated in the complex organization of mammalian development by determining how apoptotic decisions are regulated in tissues will be an area of intensive research. In the meantime, to secure a comprehensive understanding of the regulation of the conserved and divergent mechanisms of cell death regulation across species, further efforts should be continued at programmed cell death pathways in *C. elegans*, *Drosophila* and principally murine models.

References

1. **Baehrecke EH.** How death shapes life during development. *Nat Rev Mol Cell Biol*. 2002; 3: 779–87.
2. **Vaux DL, SJ Korsmeyer.** Cell death in development. *Cell*. 1999; 96: 245–54.
3. **Hipfner DR, SM Cohen.** Connecting proliferation and apoptosis in development and disease. *Nat Rev Mol Cell Biol.* 2004; 5: 805–15.
4. **Kerr JF, AH Wyllie, AR Currie.** Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cance.* 1972; 26: 239–57.
5. **Schweichel JU, HJ Merker.** The morphology of various types of cell death in prenatal tissues. *Teratology* 1973; 7: 253–66.

6. **Colussi PA, S Kumar.** Targeted disruption of caspase genes in mice: what they tell us about the functions of individual caspases in apoptosis. *Immunol Cell Biol.* 1999; 77: 58–63.
7. **Hawkins CJ, DL Vaux.** The role of the Bcl-2 family of apoptosis regulatory proteins in the immune system. *Semin Immunol.* 1997; 9: 25–33.
8. **Yeh WC, Hakem R, Woo M, Mak TW.** Gene targeting in the analysis of mammalian apoptosis and TNF receptor superfamily signaling. *Immunol Rev.* 1999; 169: 283–302.
9. **Ranger AM, BA Malynn, SJ Korsmeyer.** Mouse models of cell death. *Nat Genet.* 2001*,* 28: 113–8.
10. **Ellis HM, HR Horvitz.** Genetic control of programmed cell death in the nematode C. elegans*.* *Cell* 1986; 44: 817–29.
11. **Liu QA, MO Hengartner.** The molecular mechanism of programmed cell death in C. elegans. *Ann N Y Acad Sci.* 1999; 887: 92–104.
12. **Muqit MM, MB Feany.** Modelling neurodegenerative diseases in Drosophila: a fruitful approach? *Nat Rev Neurosci.* 2002; 3: 237–43.
13. **Abrams JM.** An emerging blueprint for apoptosis in Drosophila. *Trends Cell Biol.* 1999; 9: 435-40.
14. **Richardson H, S Kumar.** Death to flies: Drosophila as a model system to study programmed cell death. *J Immunol Methods.* 2002; 265: 21–38.
15. **Hay BA, JR Huh, M Guo.** The genetics of cell death: approaches, insights and opportunities in Drosophila. *Nat Rev Genet.* 2004; 5: 911–22.
16. **Horvitz HR.** Genetic control of programmed cell death in the nematode Caenorhabditis elegans. *Cancer Res.* 1999; 59: 1701s–6s.
17. **Horvitz HR, Sternberg PW, Greenwald IS, Fixsen W, Ellis HM.** Mutations that affect neural cell lineages and cell fates during the development of the nematode Caenorhabditis elegans. *Cold Spring Harb Symp Quant Biol.* 1983; 48: 453–63.
18. **Conradt B, HR Horvitz.** The C. elegans protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. *Cell* 1998; 93: 519–29.
19. **Reddien PW, HR Horvitz.** The engulfment process of programmed cell death in Caenorhabditis elegans. *Annu Rev Cell Dev Biol.* 2004; 20: 193–221,
20. **Jacobson MD, M Weil, MC Raff.** Programmed cell death in animal development. *Cell* 1997; 88: 347–54.
21. **Putcha GV, EM Johnson Jr.** Men are but worms: neuronal cell death in C elegans and vertebrates. *Cell Death Differ.* 2004; 11: 38–48.
22. **Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM, Mak TW.** Apaf1 is required for mitochondrial pathways of apoptosis and brain development. *Cell* 1998; 94: 739–50.
23. **Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR.** The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. *Cell* 1993; 75: 641–52.
24. **del Peso L, Gonzalez VM, Inohara N, Ellis RE, Nunez G.** Disruption of the CED-9.CED-4 complex by EGL-1 is a critical step for programmed cell death in Caenorhabditis elegans. *J Biol Chem.* 2000; 275: 27205–11.
25. **Chinnaiyan AM.** The apoptosome: heart and soul of the cell death machine. *Neoplasia* 1999; 1: 5–15.
26. **Riedl SJ, Y Shi.** Molecular mechanisms of caspase regulation during apoptosis. *Nat Rev Mol Cell Biol.* 2004; 5: 897–907.
27. **Shi Y.** Mechanisms of caspase activation and inhibition during apoptosis. *Mol Cell* 2002; 9: 459–70.
28. **Zimmermann KC, C Bonzon, DR Green,** The machinery of programmed cell death. *Pharmacol Ther.* 2001; 92: 57–70.
29. **Wajant H.** The Fas signaling pathway: more than a paradigm. *Science* 2002; 296: 1635–6.
30. **Putcha GV, Harris CA, Moulder KL, Easton RM, Thompson CB, Johnson EM Jr.** Intrinsic and extrinsic pathway signaling during neuronal apoptosis: lessons from the analysis of mutant mice. *J Cell Biol.* 2002; 157: 441–53.
31. **Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X.** Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 1997; 91: 479–89.
32. **Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, Scaffidi C, Bretz, JD, Zhang M, Gentz R, Mann M, Krammer PH, Peter ME, Dixit VM.** FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death—inducing signaling complex. *Cell* 1996; 85: 817–27.
33. **Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH, Peter ME.** FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). *EMBO J.* 1997; 16: 2794–804.
34. **Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano, CA, Newmeyer DD, Wang HG, Reed JC, Nicholson DW, Alnemri ES, Green DR, Martin SJ.** Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J Cell Biol.* 1999; 144: 281–92.
35. **Zou H, Henzel WJ, Liu X, Lutschg A, Wang X.** Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* 1997; 90: 405–13.
36. **Rodriguez A, Oliver H, Zou H, Chen P, Wang X, Abrams JM.** Dark is a Drosophila homologue of Apaf-1/CED-4 and functions in an evolutionarily conserved death pathway. *Nat Cell Biol.* 1999; 1: 272–9.
37. **Daniel PT, Schulze-Osthoff K, Belka C, Guner D.** Guardians of cell death: the Bcl-2 family proteins. *Essays Biochem.* 2003; 39: 73–88.
38. **Bouillet P, A Strasser.** BH3-only proteins - evolutionarily conserved proapoptotic Bcl-2 family members essential for initiating programmed cell death. *J Cell Sci.* 2002; 115: 1567–74.
39. **Borner C.** The Bcl-2 protein family: sensors and checkpoints for life-or-death decisions. *Mol Immunol.* 2003; 39: 615–47.

40. **Donovan M, TG Cotter.** Control of mitochondrial integrity by Bcl-2 family members and caspase-independent cell death. *Biochim Biophys Acta.* 2004*,* 1644: 133–47.
41. **Wang X, Yang C, Chai J, Shi Y, Xue D.** Mechanisms of AIF-mediated apoptotic DNA degradation in Caenorhabditis elegans. *Science* 2002; 298: 1587–92.
42. **Srinivasula SM, Datta P, Kobayashi M, Wu JW, Fujioka M, Hegde R, Zhang Z, Mukattash R, Fernandes-Alnemri T, Shi Y, Jaynes JB, Alnemri ES.** Sickle, a novel Drosophila death gene in the reaper/hid/grim region, encodes an IAP-inhibitory protein. *Curr Biol.* 2002; 12: 125–30.
43. **Wing JP, Karres JS, Ogdahl JL, Zhou L, Schwartz LM, Nambu JR.** Drosophila sickle is a novel grim-reaper cell death activator. *Curr Biol.* 2002; 12: 131–5.
44. **Salvesen GS, CS Duckett.** IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol.* 2002; 3: 401–10.
45. **Martins LM, Iaccarino I, Tenev T., Gschmeissner S, Totty NF, Lemoine NR, Savopoulos J, Gray CW, Creasy CL, Dingwall C, Downward J.** The serine protease Omi/HtrA2 regulates apoptosis by binding XIAP through a reaper-like motif. *J Biol Chem.* 2002; 277: 439–44.
46. **Cande C, Vahsen N, Garrido C, Kroemer G.** Apoptosis-inducing factor (AIF): caspase-independent after all. *Cell Death Differ.* 2004; 11: 591–5.
47. **Vaux DL, J Silke.** HtrA2/Omi, a sheep in wolf's clothing. *Cell*. 2003; 115: 251–3.
48. **Sulston JE, JG White.** Regulation and cell autonomy during postembryonic development of Caenorhabditis elegans. *Dev Biol.* 1980; 78: 577–97.
49. **Baehrecke EH,** Steroid regulation of programmed cell death during Drosophila development. *Cell Death Differ.* 2000; 7: 1057–62.
50. **Barde YA,** Trophic factors and neuronal survival. *Neuron.* 1989; 2: 1525–34.
51. **Krammer PH,** CD95's deadly mission in the immune system. *Nature* 2000; 407: 789–95.
52. **Jiang C, EH Baehrecke, CS Thummel.** Steroid regulated programmed cell death during Drosophila metamorphosis*. Development*. 1997; 124: 4673–83.
53. **Puthalakath H, A Strasser.** Keeping killers on a tight leash: transcriptional and post-translational control of the pro-apoptotic activity of BH3-only proteins. *Cell Death Differ.* 2002; 9: 505–12.
54. **Yeo W, J Gautier.** Early neural cell death: dying to become neurons. *Dev Biol.* 2004; 274: 233–44.
55. **Hidalgo A, C ffrench-Constant.** The control of cell number during central nervous system development in flies and mice. *Mech Dev.* 2003; 120: 1311–25.
56. **Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD.** Programmed cell death and the control of cell survival: lessons from the nervous system. *Science* 1993; 262: 695–700.
57. **Nijhawan D, N Honarpour, X Wang.** Apoptosis in neural development and disease. *Annu Rev Neurosci.* 2000*;* 23: 73–87.
58. **Bergmann A.** Regulation of cell number by MAPK-dependent control of apoptosis: a mechanism for trophic survival signaling. *Dev Cell* 2002; 2: 159–70.
59. **Bergmann A, Agapite J, McCall K, Steller H.** The Drosophila gene hid is a direct molecular target of Ras-dependent survival signaling. *Cell* 1998; 95: 331–41.
60. **Conradt B, HR Horvitz.** The TRA-1A sex determination protein of C. elegans regulates sexually dimorphic cell deaths by repressing the egl-1 cell death activator gene. *Cell* 1999; 98: 317–27.
61. **Cakouros D, Daish T, Martin D, Baehrecke EH, Kumar S.** Ecdysone-induced expression of the caspase DRONC during hormone-dependent programmed cell death in Drosophila is regulated by Broad-Complex. *J Cell Biol.* 2002; 157: 985–95.
62. **Dorstyn L, Colussi PA, Quinn LM, Richardson H, Kumar S.** DRONC, an ecdysone-inducible Drosophila caspase. *Proc Natl Acad Sci USA.* 1999; 96: 4307–12.
63. **Nakayama K, Negishi I, Kuida K, Sawa H, Loh DY.** Targeted disruption of Bcl-2 alpha beta in mice: occurrence of gray hair, polycystic kidney disease, and lymphocytopenia. *Proc Natl Acad Sci USA.* 1994; 91: 3700–4.
64. **Motoyama N, Wang F, Roth KA, Sawa H, Nakayama K, Nakayama K, Negishi I, Senju S, Zhang Q, Fujii S, *et al.*** Massive cell death of immature hematopoietic cells and neurons in Bcl-x-deficient mice. *Science* 1995; 267: 1506–10.
65. **Varfolomeev EE, Schuchmann M, Luria V, Chiannilkulchai N, Beckmann JS, Mett IL, Rebrikov, D, Brodianski VM, Kemper OC, Kollet O, Lapidot T, Soffer D, Sobe T, Avraham KB, Goncharov T, Holtmann H, Lonai P, Wallach D.** Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and is lethal prenatally. *Immunity* 1998; 9: 267–76.
66. **Kang TB, Ben-Moshe T, Varfolomeev EE, Pewzner-Jung Y, Yogev N, Jurewicz A, Waisman A, Brenner O, Haffner R, Gustafsson E, Ramakrishnan P, Lapidot T, Wallach D.** Caspase-8 serves both apoptotic and nonapoptotic roles. *J Immunol.* 2004; 173: 2976–84.
67. **Kuida K, Zheng TS, Na S, Kuan C, Yang D, Karasuyama H, Rakic P, Flavell RA.** Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature* 1996; 384: 368–72.
68. **Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P, Flavell RA.** Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell* 1998; 94: 325–37.